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Original Article

Genetic Associations of Mitochondrial DNA Polymorphisms with Behçet's Disease in a Korean Population: A Pilot Study

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Objective. Pathogenesis of Behçet's disease (BD) is known to be multifactorial and accumulating data suggest genetic mechanisms. Variations in nuclear DNAs have been largely investigated, while studies on mitochondrial DNAs are limited. The purpose of the current study is to investigate associations of mitochondrial single nucleotide polymorphisms and haplotypes with BD. **Methods.** Complete mitochondrial DNAs were sequenced using chip array with blood samples collected from 20 patients and 10 control subjects. Haplotypes were searched in hypervariable region 1 and 2. Chi square or Fisher's exact test was used to analyze associations of mitochondrial single nucleotide polymorphisms between two groups and associations between clinical characteristics and mitochondrial single nucleotide polymorphisms. **Results**. From a total of 16,569 for each individual, 16,545 mitochondrial DNA nucleotides were sequenced. m.248A>G, m.709G>A, m.3970C>T, m.6392T>C, m.6962G>A, m.10310G>A, m.10609T>C, m.12406G>A, m.12882C>T, m.13928G>C, m.16129G>A, and m16304T>C were observed more frequently in the patient group, although without statistical significance, while m.304C>A, m.3010G>A, m.4883C>T, m.5178C>A, and m.14668C>T were more frequent in the control group (p=0.008, 0.026, 0.007, 0.007, and 0.026, respectively). m.16182A>C, m.16183A>C, and m.16189T>C were associated with uveitis (p=0.041, 0.022, and 0.014, respectively). None of the haplotypes we searched were statistically associated with BD risk, but B4a was observed more frequently in the patient group. **Conclusion.** We report the first association study between BD and mitochondrial single nucleotide polymorphisms in a Korean population. In the current study, m.248A>G, m.709G>A, m.3970C>T, m.6392T>C, m.6962G>A, m.10310G>A, m.10609T>C, m.12406G>A, m.12882C>T, m.13928G>C, m.16129G>A, and m16304 T>C could be candidate mitochondrial single nucleotide polymorphisms in BD. **(J Rheum Dis 2016;23:23-29)**

Key Words. Behçet's disease, Mitochondria, Polymorphism, Haplotypes, Etiology

INTRODUCTION

Behçet's disease (BD) is a chronic systemic inflammatory disorder characterized by recurrent oral ulcers, genital ulcers, skin lesions and eye lesions. The prevalence of BD highly distributed to specific geographical areas including the Mediterranean and the Middle Eastern countries. Pathogenesis of BD remains to be elucidated, and various pathogenetic studies suggesting susceptibility nuclear genes have been reported so far including human leukocyte antigen (HLA)-B51, MHC class I polypeptide-related sequence A (MICA), and tumor necrosis factor (TNF) [1-3]. On the contrary, studies on mitochondrial DNAs (mtDNA) were scarcely reported. To the best of our knowledge, there is only one report in Iran, in which association of mitochondrial polymorphism m.709G>A with BD susceptibility in Iranian population was reported [4]. This is the first pilot study performed to investigate associations of mitochondrial single nucleotide polymorphisms (mtSNPs) and haplotypes with BD in a Korean population.

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MATERIALS AND METHODS

Patients

A total of 20 BD patients who met the 1999 Diagnostic Criteria of International Study Group for Behçet's disease aged between 20 and 60 were enrolled from October 2012 to March 2013 [5]. Exclusion criterion was presence of other autoimmune or autoinflammatory Diseases. Ten Korean healthy blood donors were enrolled for control group. The study protocol was approved by the Institutional Review Board of Konyang University Hospital (IRB approval no. KYUH 12-48), and adhered to the Declaration of Helsinki. All patients provided written informed consent.

Case records consisted of patients' current age; age at diagnosis of BD; family history of BD, clinical manifestations of genital ulcer, uveitis, optic neuritis, erythema nodosum-like skin lesion, pseudofolliculitis, gastrointestinal involvement, thrombosis; laboratory results including HLA-B51 positivity (Table 1).

Methods

1) DNAs extraction

After extracting genomic DNAs from peripheral blood, we performed qualitative and quantitative analyses. Then sample was diluted into 100 ng/ μL.

2) Human Mitochondrial Resequencing Array

We used GeneChip Human Mitochondrial Resequencing Array ver. 2.0 (Affymetrix Inc., Santa Clara, CA, USA) which uses eight 25-mer probes per base position varied at the central position to incorporate each possible nucleotide allowing for the detection of SNPs to cover whole human mtDNAs of approximately 16.5 kb. Polymerase chain reaction was performed with 3 mitochondrial specific primers in GeneChip DNA Resequencing Assay kit (Affymetrix Inc.) designed to amplify each of 3 long fragments with lengths of 3,968 bp, 5,513 bp, and 7,814 bp to cover the whole human mtDNAs. According to the manufacturer's protocols resequencing array was performed.

Table 1. Clinical characteristics of patients with Behçet's disease

Characteristic	Patient (n = 20)
Demographic feature	
Age (yr)	49 ± 12.54
Sex (M:F)	6:14
Duration (yr)	12 ± 6.80
HLA-B51, positive	6/20 (30)
Clinical manifestation	
Oral ulcer	20 (100)
Skin lesions	16 (80)
Genital ulcer	14 (70)
Uveitis	5 (25)
Pathergy test, positive	2 (10)

Values are presented as mean ± standard deviation or number (%) F: female, HLA: human leukocyte antigen, M: male.

3) Data analysis and statistical analysis

The Affymetrix MitoChip ver. 2.0 (Affymetrix Inc.) uses the Cambridge Reference Sequence (RCRS) as the reference sequence template, with additional 478 fragments in order to detect the approximately 500 haplotypes in the Mitomap database. And microarray data was analyzed using GeneChip Operating Software ver. 1.4 (GCOS 1.4; Affymetrix Inc) and Genechip Sequence Analysis Software (GSEQ 4.0; Affymetrix Inc). Haplotypes were searched in the hypervariable (HV) region 1, 2 with web-based software, mtDNA manager (http://mtmanager.yonsei.ac.kr/). The associations were examined by comparing frequencies of alleles or haplotypes in two groups using chi-square test or Fisher's exact test using SAS 9.1.3 (SAS Institute, Cary, NC, USA). And associations of several clinical manifestations with variations of mtDNAs of BD patients were analyzed. We calculated an odds ratio, a 95% confidence interval, and a two-tailed p-value. Values of $p<0.05$ indicated statistical significance.

RESULTS

Results of chip microarray

Sixteen thousand five hundred forty five nucleotides were sequenced from total of 16,569 nucleotides for each individual according to experimental design which could not sequence 12 nucleotides at each ends. Overall detection call rate was approximately 97.5% at quality score of 9. Among 16,545 sequenced mitochondrial DNA nucleotides, 10 nucleotide alterations were uniformly observed in all BD patients and healthy controls compared from reference sequences; m.73A>G, m.263A>G, m.750A>G, m.2706A>G, m.4769A>G, m.7028C>T, m.8860A>G, m.11719G>A, m.14766C>T, and m.15326A>G. On the other hand 16,250 nucleotides in all subjects were completely concordant with reference sequences. Therefore 285 nucleotide alterations were included for further analysis. Figure 1 shows part of chip ar-

ray results, from which omitted mtDNA variations occurred with similar frequencies in both groups.

mtSNP analysis of whole mitochondrial DNAs for BD susceptibility

There were no mtSNPs associated with BD susceptibility

RCRS Mitochondira Position	Map Locus	Map Position(np)	Gene Location	Description	RCRS Reference Sequence	SNP Count	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	P01	P02	P03	P04	P05	P06	P07	P08	P09	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20
146	MT-HV2	57-372	HVS2/HV2	Hypervariable segment 2	t	3	-	-	-	-	-	-	-	-	-	-	c	-	-	-	-	-	-	-	-	-	c	-	-	-	-	-	c	-	-
150	MT-HV2	57-372	HVS2/HV2	Hypervariable segment 2	c	6	t	t	-	-	t	-	t	-	-	-	-	-	-	-	-	-	t	-	-	-	t	-	-	-	-	-	-	-	-
200	MT-HV2	57-372	HVS2/HV2	Hypervariable segment 2	a	4	-	t	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	g	-	-	-	-	-	g	g	-	-	-
248	MT-HV2	57-372	HVS2/HV2	Hypervariable segment 2	a	6	-	-	-	-	-	-	-	-	-	-	g	-	g	g	-	g	-	-	-	-	-	g	-	-	-	-	-	-	g
304	MT-HV2	57-372	HVS2/HV2	Hypervariable segment 2	c	4	-	a	-	-	a	a	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
307	MT-HV2	57-372	HVS2/HV2	Hypervariable segment 2	c	3	-	-	-	-	a	-	a	-	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
709	MT-RNR1	648-1601	12S	12S ribosomal RNA	g	6	-	-	-	-	-	-	-	a	-	-	-	-	-	-	a	-	-	-	-	a	-	-	a	-	-	a	-	a	-
3010	MT-RNR2	1671-3229	16S	16S ribosomal RNA	g	7	-	-	-	a	-	a	a	a	-	a	-	-	-	-	-	-	-	-	-	-	-	a	-	-	-	-	a	-	-
3970	MT-ND1	3307-4262	ND1	NADH Dehydrogenase subunit 1	c	5	-	-	-	-	-	-	-	-	-	-	-	-	t	t	-	t	-	-	-	-	-	t	-	-	-	-	-	-	t
4086	MT-ND1	3307-4262	ND1	NADH Dehydrogenase subunit 1	c	3	-	-	-	-	-	-	-	-	-	-	-	-	t	-	-	t	-	-	-	-	-	-	-	-	-	-	-	-	t
5178	MT-ND2	4470-5511	ND2	NADH dehydrogenase subunit 2	c	8	-	-	-	a	a	a	a	a	-	a	-	-	-	-	-	-	-	-	-	-	-	a	-	-	-	-	a	-	-
5465	MT-ND2	4470-5511	ND2	NADH dehydrogenase subunit 2	t	3	-	-	-	-	-	-	-	-	-	-	-	c	-	-	-	-	-	-	-	c	-	-	-	-	-	-	-	c	-
6392	MT-CO1	5904-7445	COI	Cytochrome c oxidase subunit I	t	5	-	-	-	-	-	-	-	-	-	-	-	-	c	c	-	c	-	-	-	-	-	c	-	-	-	-	-	-	c
6962	MT-CO1	5904-7445	COI	Cytochrome c oxidase subunit I	g	5	-	-	-	-	-	-	-	-	-	-	-	-	a	a	-	a	-	-	-	-	-	a	-	-	-	-	-	-	a
8414	MT-ATP8	8366-8572	ATPase8	ATP synthase F0 subunit 8	c	6	-	-	-	t	-	t	-	t	-	t	-	-	-	-	-	-	-	-	-	-	-	-	t	-	-	-	-	t	-	-
8584	MT-ATP6	8527-9207	ATPase6	ATP synthase F0 subunit 6	g	3	-	-	-	-	-	-	-	-	-	-	a	-	-	-	a	-	-	-	-	-	-	-	-	a	-	-	-	-	-	-
9053	MT-ATP6	8527-9207	ATPase6	ATP synthase F0 subunit 6	g	3	-	-	-	-	-	-	-	-	-	-	-	-	a	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	a
9123	MT-ATP6	8527-9207	ATPase6	ATP synthase F0 subunit 6	g	3	-	-	-	-	-	-	-	-	-	-	-	a	-	-	-	-	-	-	-	a	-	-	-	-	-	-	-	a	-
10310	MT-ND3	10059-10404	ND3	NADH dehydrogenase subunit 3	g	6	-	-	-	-	-	-	-	-	a	-	-	-	a	a	-	a	-	-	-	-	-	a	-	-	-	-	-	-	a
10400	MT-ND3	10059-10404	ND3	NADH dehydrogenase subunit 3	c	10	-	-	-	-	-	-	-	t	-	t	t	-	-	-	-	-	t	-	-	-	t	-	-	t	t	t	t	t	-	-
10609	MT-ND4L	10470-10766	ND4L	NADH dehydrogenase subunit 4L	t	5	-	-	-	-	-	-	-	-	-	-	-	-	c	c	-	c	-	-	-	-	-	c	-	-	-	-	-	-	c
12358	MT-ND5	12337-14148	ND5	NADH dehydrogenase subunit 5	a	2	-	g	-	-	-	-	g	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12406	MT-ND5	12337-14148	ND5	NADH dehydrogenase subunit 5	g	5	-	-	-	-	-	-	-	-	-	-	-	-	a	a	-	a	-	-	-	-	-	a	-	-	-	-	-	-	a
12882	MT-ND5	12337-14148	ND5	NADH dehydrogenase subunit 5	c	5	-	-	-	-	-	-	-	-	-	-	-	-	t	t	-	t	-	-	-	-	-	t	-	-	-	-	-	-	t
13759	MT-ND5	12337-14148	ND5	NADH dehydrogenase subunit 5	g	3	-	-	-	-	-	-	-	-	-	-	-	-	a	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	a
13928	MT-ND5	12337-14148	ND5	NADH dehydrogenase subunit 5	g	5	-	-	-	-	-	-	-	-	-	-	-	-	c	c	-	c	-	-	-	-	-	c	-	-	-	-	-	-	c
14668	MT-ND6	14149-14673	ND6	NADH dehydrogenase subunit 6	c	7	-	-	-	t	-	t	t	t	-	t	-	-	-	-	-	-	-	-	-	-	-	-	t	-	-	-	-	t	-	-
16129	MT-HV1	16024-16383	HVS1/HV1	Hypervariable segment 1	g	8	a	-	-	-	-	-	-	-	-	-	-	-	a	a	a	-	a	-	-	-	a	-	-	a	-	-	-	-	-	a
16184	MT-HV1	16024-16383	HVS1/HV1	Hypervariable segment 1	c	3	-	-	-	t	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	t	-	-	-	-	-
16245	MT-HV1	16024-16383	HVS1/HV1	Hypervariable segment 1	c	3	-	t	-	-	-	-	-	t	-	-	-	-	-	-	-	-	-	-	-	-	-	-	t	-	-	-	-	-	-	-
16304	MT-HV1	16024-16383	HVS1/HV1	Hypervariable segment 1	t	6	c	-	-	-	-	-	-	-	-	-	-	-	c	c	-	c	-	-	-	-	-	c	-	-	-	-	-	-	-	c

Figure 1. Results of part of chip array. Mitochondrial alterations with different frequencies between two groups are shown. COI: cytochrome c oxidas, HV: hypervariable region, MT: mitochondria, ND: NADH dehydrogenase, RCRS: Cambridge Reference Sequence, SNP: single nucleotide polymorphisms.

Table 2. Associations between mitochondrial DNA alterations and Behçet's disease (BD)

RCRS position	Alteration	BD (n=20)	Control (n=10)	p-value*	OR* (95% CI)
248	A>G	6 (30)	0 (0)	0.074	NA
304	C>A	0 (0)	4 (40)	0.008	NA
709	G>A	5 (25)	1 (10)	0.633	3.000 (0.301~29.940)
3010	G>A	2 (10)	5 (50)	0.026	0.111 (0.016~0.755)
3970	C>T	5 (25)	0 (0)	0.140	NA
4883	C>T	2 (10)	6 (60)	0.007	0.074 (0.011~0.512)
5178	C>A	2 (10)	6 (60)	0.007	0.074 (0.011~0.512)
6392	T>C	5 (25)	0 (0)	0.140	NA
6962	G>A	5 (25)	0 (0)	0.140	NA
10310	G>A	5 (25)	1 (10)	0.633	3.000 (0.301~29.940)
10609	T>C	5 (25)	0 (0)	0.140	NA
12406	G>A	5 (25)	0 (0)	0.140	NA
12882	C>T	5 (25)	0 (0)	0.140	NA
13928	G>C	5 (25)	0 (0)	0.140	NA
14668	C>T	2 (10)	5 (50)	0.026	0.111 (0.016~0.755)
16129	G>A	7 (35)	1 (10)	0.210	4.846 (0.505~46.492)
16304	T>C	5 (25)	1 (10)	0.633	3.000 (0.301~29.940)

Values are presented as number (%). CI: confidence interval, NA: non-applicable, OR: odds ratio, RCRS: Cambridge Reference Sequence. *Fisher's exact test.

with statistical significance between BD patients and controls. However, m.248A>G, m.709G>A, m.3970C>T, m.6392T>C, m.6962G>A, m.10310G>A, m.10609T>C, m.12406G>A, m.12882C>T, m.13928G>C, m.16129G>A, and m16304T>C were more frequently observed in the BD group. While, m.304C>A, m.3010G>A, m.4883C>T, m.5178C>A, and m.14668C>T were more frequent in the control group with statistical significance (p=0.008, 0.026, 0.007, 0.007, and 0.026, respectively) (Table 2).

mtSNPs analysis and clinical/laboratory features

Clinical variables consisted of presence of deep, multiple oral ulcers, posterior location, genital ulcer, erythema nodosum, pseudofolliculitis, uveitis, optic neuritis, thrombus, arthralgia/arthritis, pathergy test, vasculitis, gastrointestinal involvement. Laboratory variables were ANA, rheumatoid factor, HLA-B27, and HLA-B51. By Fisher's exact test, m.16182A>C, m.16183A>C, and m.16189T>C were associated with uveitis (p=0.041, 0.022, and 0.014, respectively). The location of all of three mtDNAs is in the HV region 1.

Haplotype analysis

Haplotypes were searched in the HV region 1 and 2 with web-based software, mtDNA manager (http://mtmanager.yonsei.ac.kr/). In these non-coding regions located in the D-loop, nucleotide alterations such as substitutions or deletions are very frequently found than in other sites. We found no significant association of haplotypes in the HV regions with BD (Table 3). B4a was found in 3 patients and none in control, but there was no statistical difference by Fisher's exact test (p=0.532).

DISCUSSION

BD is a rare chronic systemic inflammatory disorder characterized with recurrent oral ulcer, genital ulcer, eye and skin lesions. Major pathologic finding is vasculitis affecting various kinds and sizes of vessels and involvement of various internal organs such as central nervous system, lungs, gastrointestinal tracts, kidneys have been known besides characteristic manifestations. BD simultaneously has autoimmune and autoinflammatory characteristic, the exact etiopathogenesis is not fully understood. Generally, it is hypothesized that BD could develop when environmental triggers affect individuals with genetic susceptibility. Based on disclosed clinical, laboratory and biochemical findings, many nuclear genes have been suggested as susceptibility genes for BD. HLA-B51 is the most established susceptibility gene, and several other HLA class I and class II alleles have been described to be associated with BD in different populations [1]. Studies suggesting associations of polymorphisms of genes encoding cytokines such as interleukins, TNF and BD are growing [2]. On the contrary, studies on abnormalities of mitochondrial genes are limited. There is one short report of mtSNPs for susceptibility of BD performed in Iran, reporting association of m.709G>A with BD [4].

Human mitochondrial DNA is a circular, covalently closed, double-stranded DNA located in mitochondria. A set of mitochondrial DNA consists of 16,569 nucleotides, and it encodes 37 genes. Main function of mitochondria is as a power plant, making energy from respiratory chain by transfer of electrons from nicotinamide adenine dinucleotide reduced form (NADH) to coenzyme Q10. On the other hand, mitochondria also produce reactive oxygen species increasing cellular oxidative stress which is linked to neuromuscular diseases and aging [6]. Mitochondrial disorder is a heterogenous entity primarily manifested with either neuropathic or myopathic features, however, it is currently an expanding clinical spectrum with more emerging mitochondrial disorders and several existing disorders are explained with mitochondrial hypotheses [7-9]. Mitochondrial disorders could present as a single organ disorder but many of them involve multiple organs and tissues and BD is a multigenetic disorder with systemic involvement. BD is an autoinflammatory disorder of which mechanism is inflammasome-mediated hyperactivation of innate immunity and currently, interregulations between mitochondria and inflammasomes have been suggested [10,11]. Thus, we could hypothesize that mitochondrial abnormalities from mtSNPs could be associated with BD susceptibility.

This study is the first association study searching for mtSNPs for BD susceptibility in a Korean population. To the best of our knowledge, it is the first study to identify the whole mitochondrial genes of 30 Korean individuals including 20 BD patients. Scarcely having information of previous results of mitochondrial genes related to BD, we decided to identify mtDNA alterations from whole sequences. As a pioneer study, we enrolled total of 20 patients and 10 healthy controls. From the overall sequencing results, ten nucleotide alterations uniformly observed in both groups compared with reference sequences may represent determinant Korean ethnicity and some of

Table 3. Results of haplotype analysis*

Sample ID	HV1, HV2 (haplotype)	Expected haplogroup	Estimated haplogroup
C01	73G 150T 199C 263G 291.1A 291.2A 310C 315.1C 315.2C 315.3C 489C 16129A 16189C 16193.1C 16223T 16297C 16298C 16302C 16304C	M7b2	M7b2
C02	73G 150T 200T 263G 291.1A 291.2A 304A 308D 315.1C 315.2C 315.3C 16145A 16172C 16193.1C 16223T 16245T 16257A 16259A 16261A 16311C 16316T 16317C 16319A	N9a4	
C03	73G 263G 291.1A 291.2A 305A 310C 315.1C 315.3C 499A 16136C 16183C 16189C 16193.1C 16193.2C 16217C 16311C 16519C	D5a1	M-489
C04	73G 263G 291.1A 291.2A 308D 310C 315.1C 315.2C 315.3C 489C 16184T 16223T 16311C	D4j3	M-489
C05	73G 150T 263G 291.1A 291.2A 304A 305A 306A 307A 310C 315.1C 315.2C 315.3C 489C 16092C 16164G 16172C 16182C 16183D 16184A 16185A 16186A 16187A 16189C 16193.1C 16223T 16261A 16262A 16263A 16266T 16267A 16276C 16311C 16519C	D5a2	M-489
C06	73G 152C 263G 291.1A 291.2A 304A 305A 306A 308D 310C 315.1C 315.2C 315.3C 489C 16174T 16193.1C 16223T 16311C 16317G	D4h	M-489
C07	73G 150T 194T 263G 291.1A 304A 305A 306A 307A 308T 309D 315.1C 489C 16188T 16193.1C 16223T 16311C 16519C	D5a1	M-489
C08	73G 263G 291.1A 291.2A 315.1C 315.2C 315.3C 489C 16190T 16224C 16245T 16292T 16362C 16519C	D4c	D4c
C09	73G 263G 291.1A 291.2A 309.3C 310C 315.1C 315.2C 315.3C 16086C 16182C 16183C 16189C 16193.1C 16217C 16311C 16519C	B4c1a	B4c1a
C10	73G 152C 263G 291.1A 291.2A 309.1C 315.1C 315.2C 315.3C 489C 16223T 16311C 16519C	M-489	M-489
P01	73G 146C 248G 263G 291.1A 291.2A 305A 306A 307A 315.1C 315.3C 489C 16193.1C 16223T 16298C 16311C 16317C 16327T 16519C	M8a1	M8
P02	73G 263G 291.1A 291.2A 310C 315.1C 315.2C 315.3C 16154C 16182C 16183C 16189C 16193.1C 16193.2C 16217C 16261T 16311C 16519C	B4a	B4a
P03	73G 152C 248G 263G 291.1A 291.2A 306A 310C 315.1C 315.2C 315.3C 548T 16129A 16162G 16172C 16304C 16311C 16519C	H1a	H1a
P04	73G 152C 248G 263G 291.1A 291.2A 305A 309.1C 310C 315.1C 315.2C 315.3C 16129A 16182C 16183C 16189C 16193.1C 16232A 16249C 16304C 16311C 16519C	D6c	
P05	73G 93G 210G 263G 291.1A 291.2A 305A 315.1C 315.2C 315.3C 16129A 16140C 16187T 16189C 16193.1C 16266A 16311C 16519C	B5a2	B5a2
P06	73G 248G 263G 291.1A 291.2A 305A 315.1C 315.2C 315.3C 16172C 16193.1C 16284G 16304C 16311C 16390A 16519C		
P07	73G 150T 199C 263G 291.1A 291.2A 310C 315.1C 315.2C 315.3C 489C 16129A 16189C 16223T 16297C 16298C 16311C	M7b2	M7b2
P08	73G 263G 291.1A 291.2A 315.1C 315.2C 315.3C 489C 16193.1C 16223T 16234T 16311C 16316G	M9a	M49
P09	73G 152C 200G 263G 291.1A 291.2A 308D 309.1C 310C 315.1C 315.2C 456T 16193.1C 16223T 16290T 16311C 16316T 16317C 16319A	H1d	
P10	73G 185A 263G 291.1A 291.2A 315.1C 315.2C 315.3C 16048A 16182C 16183C 16188.1C 16189C 16193.1C 16217C 16222T 16261T 16311C 16317C 16325C 16519C	B4a	B4a
P11	73G 150T 199C 263G 291.1A 291.2A 305A 306A 309.1C 309.2C 310C 315.1C 315.2C 315.3C 489C 16129A 16189C 16193.1C 16223T 16297C 16298C 16302C 16311C	M7b2	M7b2
P12	73G 146C 248G 263G 291.1A 291.2A 305A 309.2C 309.3C 310C 315.1C 315.2C 315.3C 16172C 16189C 16193.1C 16304C 16309G 16311C 16519C	M7e R9b1	R9b1
P13	73G 194T 199C 207A 263G 291.1A 291.2A 305A 309.1C 309.2C 309.3C 315.1C 315.2C 315.3C 489C 16193.1C 16245T 16311C	R30a	R30a
P14	73G 94A 263G 291.1A 291.2A 305A 306A 315.1C 315.2C 315.3C 489C 16129A 16176T 16193.1C 16223T 16311C 16519C	M10	M-489
P15	73G 263G 291.1A 291.2A 315.1C 315.2C 489C 16184T 16189C 16193.1C 16223T 16298C 16311C 16319A	M8a2	M8a2
P16	73G 200G 215G 263G 291.1A 306A 308D 316D 317D 318C 326G 489C 16223T 16311C	M11 R30	M11
P17	73G 143A 152C 200G 263G 291.1A 291.2A 309.1C 309.2C 309.3C 310C 315.1C 315.2C 315.3C 489C 16189C 16193.1C 16223T 16274A 16311C 16316T 16317C 16319A	G3a	M31a1
P18	73G 146C 263G 291.1A 291.2A 305A 315.1C 315.2C 315.3C 489C 16223T 16311C	M-489	M-489
P19	73G 193G 263G 291.1A 291.2A 310C 315.1C 315.2C 315.3C 16182C 16183C 16189C 16193.1C 16217C 16261T 16299G 16302C 16311C 16519C	B4a	B4a
P20	73G 248G 263G 291.1A 291.2A 315.1C 315.2C 315.3C 548T 16129A 16162G 16172C 16304C 16311C 16519C	H1a	H1a

HV: hypervariable region. *Haplotypes were searched in the HV region 1 and 2.

them identifiable in the literature were identical with the results [12]. Because of the small number of subjects it was anticipated that it is hard to get statistical significance with our results that we compared the frequency of the mtDNA alterations. As shown in Table 2, 12 mtDNA alterations were more frequently observed in patient group than control group without statistical significance.

In the previous Iranian report with 615 patients and 434 controls, m.709A>G was observed in 19.6% of BD patients by International Criteria for Behçet's Disease (ICBD) cases, 21.4% of BD patients by interferon-stimulated genes (ISG) cases and 14.5% of control (p=0.038, and 0.007) [4]. In the present study m.709A>G was observed in 5 BD patients and 1 control (p=0.633). Although there was not statistical significance, more frequent alteration was shown.

From the results of mtSNP analysis on clinical manifestations, 3 mtDNA alterations, m.16182A>C, m.16183 A>C and m.16189T>C associated with uveitis are located very adjacently together. All three mtDNA alterations are contained in haplotypes in HV 1 region, B4a and D5a.

In the present study, m.248A>G, m.709G>A, m.3970C>T, m.6392T>C, m.6962G>A, m.10310G>A, m.10609T>C, m.12406G>A, m.12882C>T, m.13928 G>C, m.16129G>A, and m16304T>C were more frequently observed in patient group. m.248A>G is a non-coding variant in locus MT-HV2, and m.16129G>A and m.16304T>C are also non-coding variants in locus MT-HV1. HV regions have far more frequent alterations of nucleotides than any other reserved regions. m.709 G>A is in locus MT-RNR1 for 12S ribosomal RNA which is also a non-coding region. m.3970C>T, m.10310G>A, m.10609T>C, m.12406G>A, m.12882C>T, and m.13928G>C are alterations in MT-ND1, MT-ND3, MT-ND4L and MT-ND5 loci, the coding regions for NADH dehydrogenase subunits. NADH dehydrogenase is the first and largest enzyme of mitochondrial electron transport chain. Other two energy-transducing enzymes involved in electron transport chain are cytochrome c reductase and cytochrome c oxidase. m.6392T>C and m.6962G>A are alterations in locus MT-COI for cytochrome c oxidase subunit I. Further investigation is required to determine if these mtDNA alterations result in dysfunction of mitochondrial electron transport or in increase of superoxide formation. And if so, how it could be linked to BD susceptibility should be elucidated.

The limitation of this study is small number of enrolled subjects to have validity and statistical conviction. And those points that mtDNAs are known to be more easily altered than those of nuclear genes and mtDNA alterations could be consequences rather than the causes of certain diseases are making researchers more difficult to translate results.

CONCLUSION

In conclusion, this is the first association study between BD and mtDNA alterations in a Korean population, and although statistically insignificant, m.248A>G, m.709 G>A, m.3970C>T, m.6392T>C, m.6962G>A, m.10310 G>A, m.10609T>C, m.12406G>A, m.12882C>T, m.13928G>C, m.16129G>A, and m16304T>C could be candidates for BD susceptibility mtSNPs. And uveitis seems to be associated with m.16182A>C, m.16183A>C, and m.16189T>C. No associated haplotype were found in HV region 1 and 2.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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